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PROJECT TITLE: BIOLOGICAL METHODS DEVELOPMENT AND UTILIZATION

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I. V79 INHIBITION OF METABOLIC COOPERATION (IMC) ASSAY

Several experiments were conducted in an effort to reinstitute the IMC assay using a lower passage cell line. As is usual, the new cell cultures were evaluated to determine their plating efficiency and response to positive control compounds. Three assays were completed. Analyses of the results indicated that the assay was functioning normally and routine testing has begun [1].

The IMC activity of hydroquinone (HQ) was evaluated [2] using a concentration range of 0.05 to 3.0 ug/ml. As with three previous experiments involving HQ, toxicity was indicated at the higher HQ concentrations. Preliminary analysis of the data indicates that dose-related activity existed for the lower concentrations. Statistical evaluations are in progress to determine the specific activity of HQ and will be reported next month.

II. SOS CHROMOTEST

A previous SOS Chromotest experiment performed in-house [3] using freshly smoked CSC from six model cigarettes indicated that none of the CSCs were active, either with or without metabolic activation (S9). However, these results were questionable due to the unknown efficacy of the lyophilized (+) S9 system supplied with the kit.

In order to test whether the lack of activity was due to the lyophilized S9 or was actually a real observation, the activity of freshly-smoked 2R1 CSC was tested using both the lyophilized S9 and the frozen S9 preparation which is routinely used in-house with S/M assays [4]. Equivalent protein concentrations were used with the two S9 systems. CSC concentrations used were comparable to those used with the previous experiment. The preliminary results indicated that while there appears to be no CSC activity using the lyophilized S9, definite activity was detected when using our standard frozen S9 preparation.

Toxicity was evaluated by measuring the enzymatic activity of alkaline phosphatase (produced by the bacteria) which is not under the control of the SOS functions. The results indicated no toxicity existed when using the frozen S9, but possible toxicity existed with the lyophilized S9 at the higher CSC concentrations.

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Experiments are being conducted to confirm these preliminary observations and will be reported next month.

III. PLANT GROWTH REGULATOR (PGR) DETERMINATIONS

An Elisa assay was conducted this month to detect the concentrations of abscisic acid (ABA) in aqueous-methanol plant extracts [5]. Comparison of the results from a standardization curve generated using a range of pure ABA concentrations with that published in the methods section of the kit indicated that the kit was working well in our hands.

To test the utility of the kit with our plant samples four samples of lamina (greenhouse senesced, greenhouse green (two different samples), and field green) were extracted using a method developed by Karen Sherwood. Several serial dilutions of the extracts were analyzed using the Elisa method. The results indicated that the concentrations of ABA in the dilutions were too high to allow an accurate determination. Plans call for preparing several more dilutions and analyzing these with the assay.

In an attempt to determine if the plant extract would interfere with the Elisa measurement of ABA, pure ABA was added to an extract dilution and analyzed using the Elisa method. The preliminary results from that experiment indicated that the plant extract does not inhibit the detection and quantitation of ABA.

IV. REFERENCES

1. Tickle, M. H. PM Notebook 8200, pp. 136.

2. Horn, J. L. PM Notebook 8229, pp. 44-51.

3. Horn, J. L. PM Notebook 8209, pp. 123-125.

4. Horn, J. L. PM Notebook 8229, pp. 52-60.

5. Davies, B. D. Notebook 8005, pp. 189-190.

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